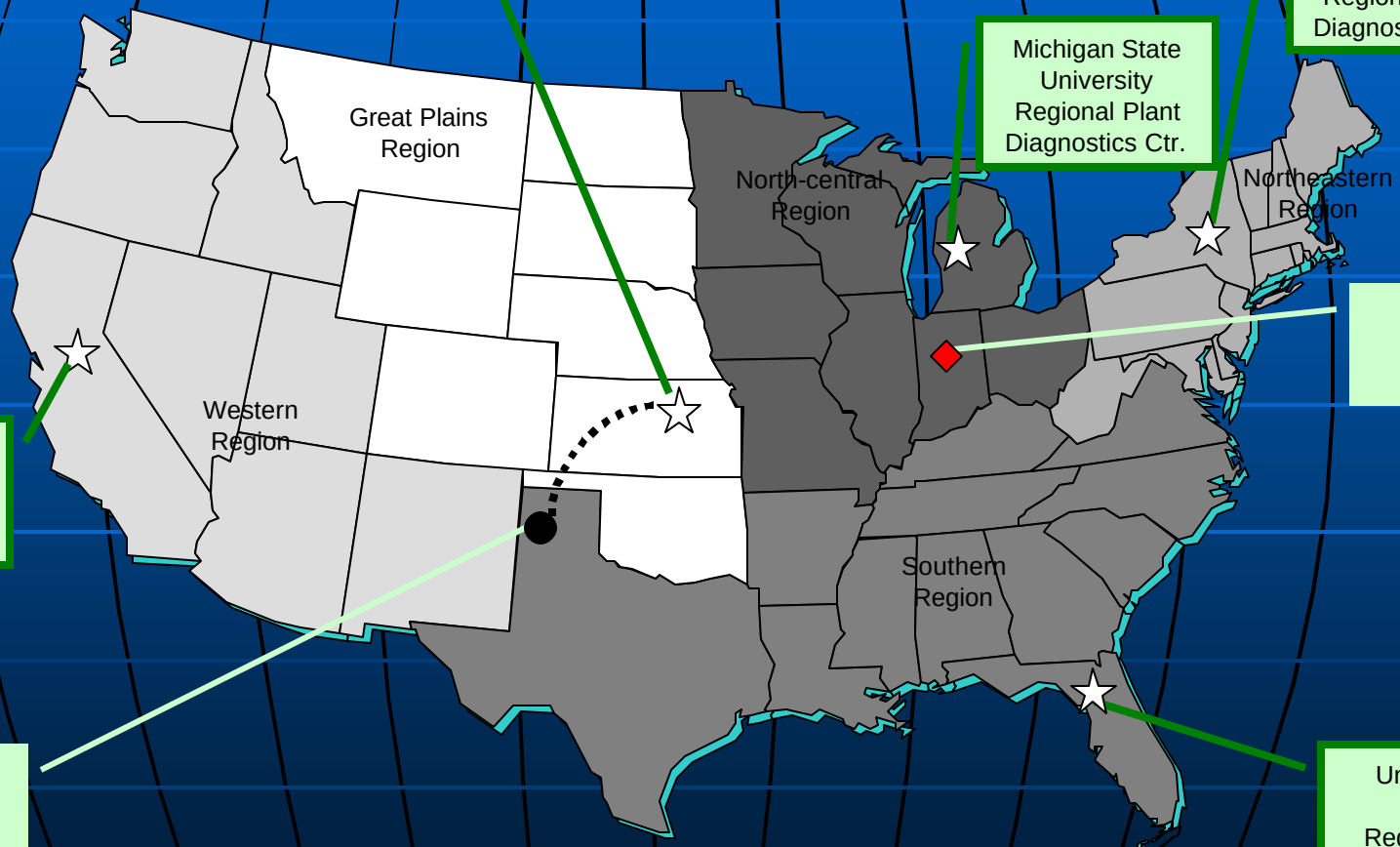
National Plant Diagnostic Network (NPDN)

Kitty Cardwell, National Program Leader
Plant Pathology
Cooperative State Research, Extension & Education Service (CSREES), USDA
Washington, DC

National Plant Diagnostic Network (NPDN)



Kansas State University Regional Plant Diagnostic Ctr.

Cornell University Animal-Satellite Regional Plant Diagnostics Ctr.

Michigan State University Regional Plant Diagnostics Ctr.

Purdue University NAPIS

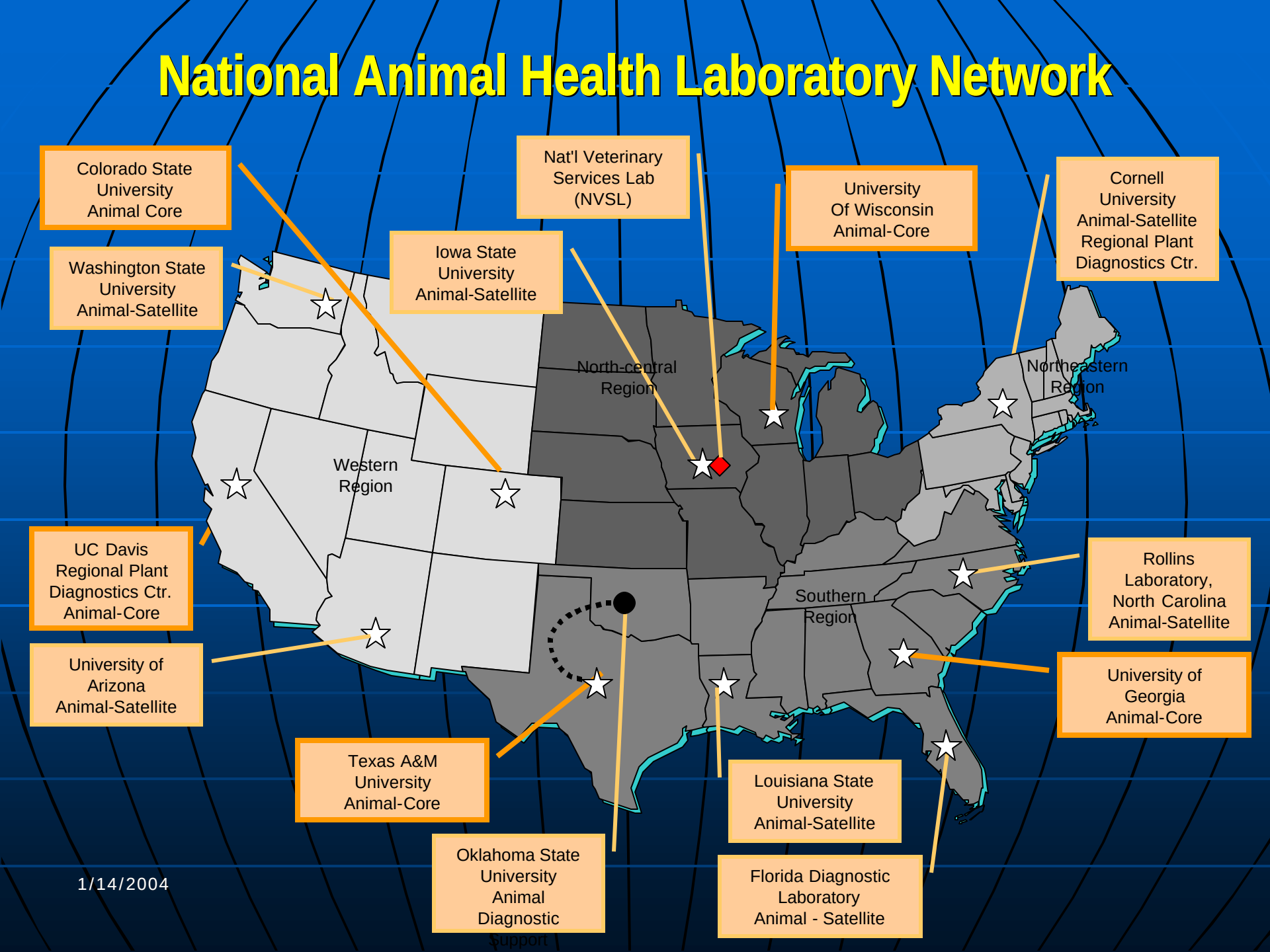
UC Davis Regional Plant Diagnostics Ctr. Animal-Core

Texas Tech University Plant Diagnostic Support

University of Florida Regional Plant Diagnostics Ctr.

1/14/2004

National Animal Health Laboratory Network



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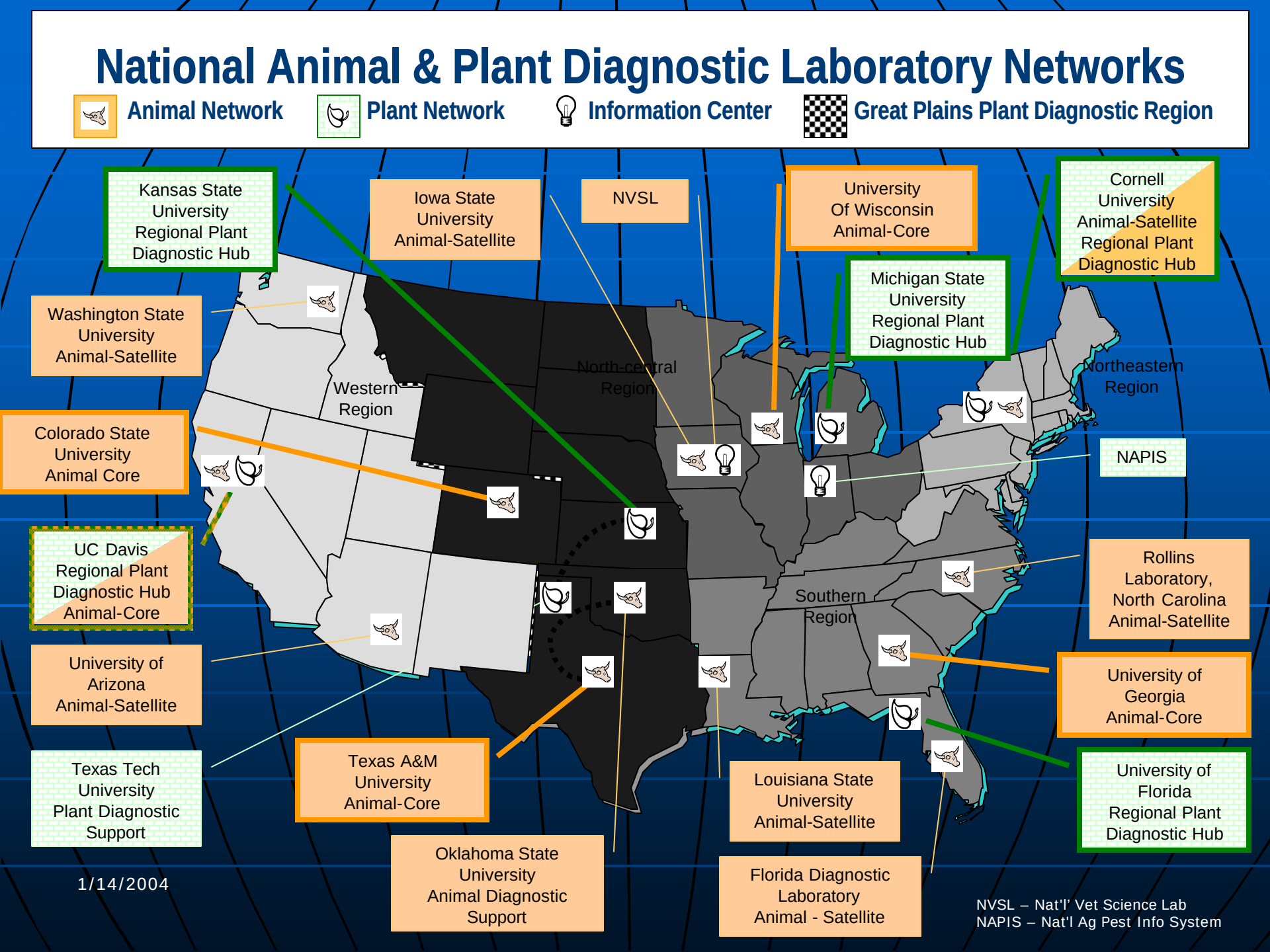
National Animal & Plant Diagnostic Laboratory Networks

Animal Network | Plant Network | Information Center | Great Plains Plant Diagnostic Region



1/14/2004

NVSL – Nat'l' Vet Science Lab
NAPIS – Nat'l Ag Pest Info System

Overall Objective

- To establish a functional national network of existing diagnostic laboratories to rapidly and accurately detect and report pathogens of national interest

National Plant Diagnostic Network

Coordination and Governance

- National Operations Committee:
  - Members
    - 2-3 per regional center
    - CSREES and APHIS personnel
  - Representatives
    - IPM centers
    - Industry - ASTA and Crop Life
    - Crop Consultants Association
    - National Plant Board
    - Emergency Disaster Extension Network

National Plant Diagnostic Network

Objectives for the 5 Regional Plant Diagnostic Centers & NAPIS

- Coordinate activities with stakeholders within regions and across the country
- Coordinate regional detection and diagnostic resources
- Train first detectors and educate public
- Establish national standard operating procedures for diagnostics, sampling and reporting
- Provide secured communications & tracking
- Create a national database to monitor pest outbreak
- Create a data analysis system to detect anomalies

National Plant Diagnostic Network

Coordinate regional detection and diagnostic resources (Education and Training Committee)

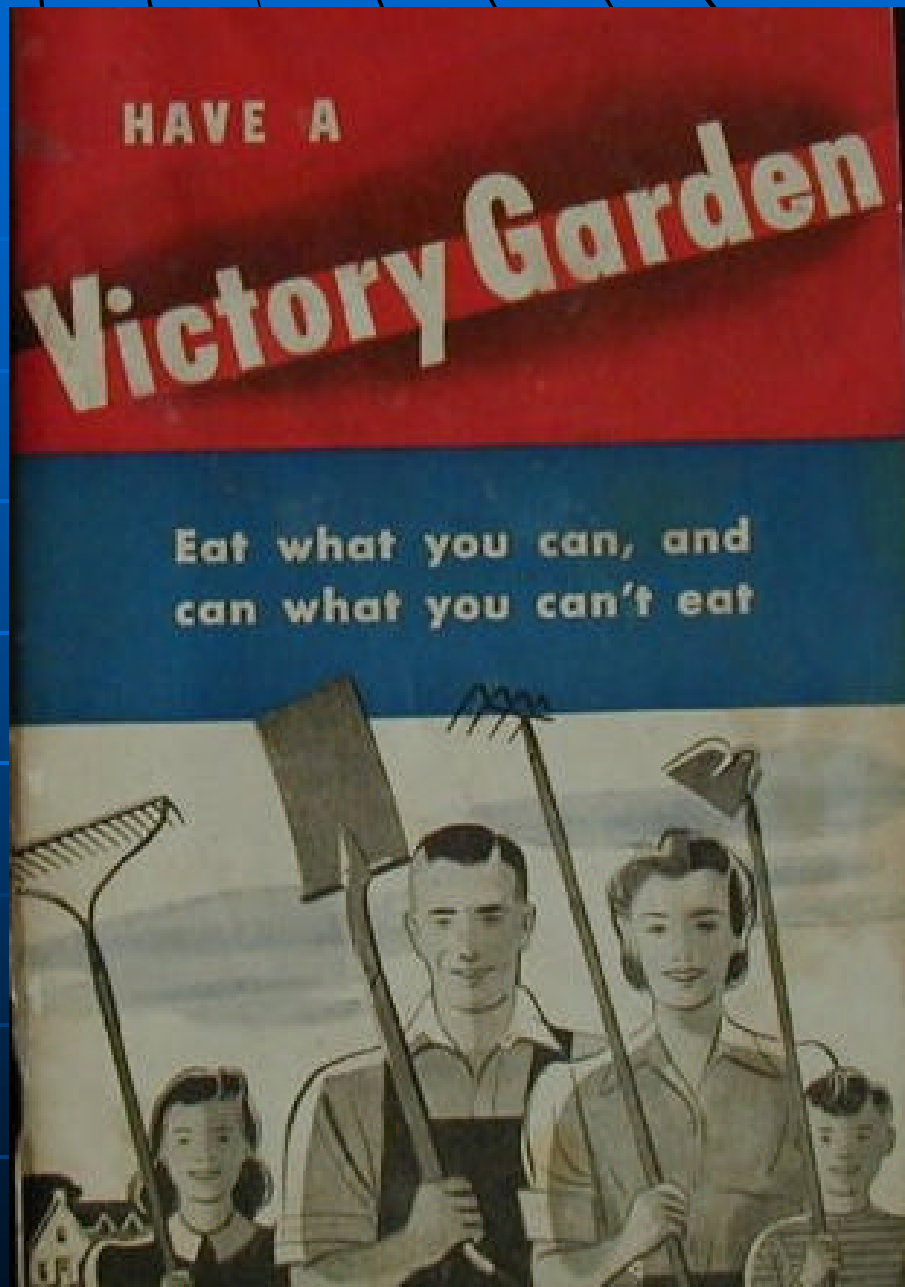
- www.npdn.org
- www.pdis.org
- www.pmn.org

National Plant Diagnostic Network

How do we educate the public?

Who do we educate?

- County Agents
- Crop Consultants
- Growers
- Master Gardeners
- State Diagnosticians
- Nat. Res. Managers
- Chemical, Seed Industry
- Extension Specialists
- Key Stakeholders
- General Public!!!



Objectives: What do we want target audience to learn?

- Increased awareness of NPDN mission
- How to recognize something new
- How to respond to an unusual find
- How to submit a quality and secure sample
- Proper use of digital imaging for diagnosis
- How/what to train others

Establish national standard operating procedures for diagnostics, sampling and reporting (Diagnostics committee)

- SOPs
- Picture Cues for Diagnosticians

National Plant Diagnostic Network

Select Agents

- ***Liberobacter africanus***, Greening, phytoplasma disease of citrus ***Liberobacter asiaticus***
- ***Peronosclerospora philippinensis***, Philippine downy mildew of corn
- ***Phakopsora pachyrhizi***, Soybean rust
- **Plum pox potyvirus**, Plum pox
- ***Ralstonia solanacearum*** Race 3 Biovar 2, Southern Wilt, Brown Rot
- ***Sclerophthora rayssiae* var. *zeae***, Brown stripe downy mildew
- ***Synchytrium endobioticum***, Potato wart or canker, host: Solanaceous plants with potato being the only cultivated host
- ***Xanthomonas oryzae* pv*. oryzicola***, Bacterial leaf streak of rice
- ***Xylella fastidiosa***, citrus variegated chlorosis strain

NPDN National Plant Diagnostic Network

Standard Operating Procedures for Plant Diagnostic Laboratories

Ralstonia solanacearum Race 3 Biovar 2

NPDN National Plant Diagnostic Network

GPDN Great Plains Diagnostic Network | NCPDN North Central Plant Diagnostic Network | NEPDN Northeast Plant Diagnostic Network | SPDN Southern Plant Diagnostic Network | WPDN Western Plant Diagnostic Network

Background:

Southern bacterial wilt has re-emerged as a disease of concern, especially to geranium growers (Williamson et al, 2001, 2002; Kim et al, 2002, Kim et al, 2003). This disease has been described in the past (Hayward, 1991; Hayward and Hartman, 1995; Strider et al, 1981; Strider, 1982), but it has recently s. It is caused by a bacterium, *Ralstonia* ...ae *solanacearum*. This is one of two ...growers are much more familiar with bacterial ...*ni (Xcp)*. *Ralstonia solanacearum* is less host- ...ect plants in the Geraniaceae (including *Pelargoniums* and hardy geraniums).

Background

Different races of *R. solanacearum* have different host ranges, including one called Race 1 that can affect many flowering crops (see Table)(Harris, 1972; Denny and Hayward, 2001). A number of weeds are known to serve as symptomless carriers of *R. solanacearum*. *Ralstonia solanacearum* is also typed according to variations in metabolic activity into 5 or 6 different biovars. Earlier descriptions of *R. solanacearum* on geraniums (Strider 1981, 1982) probably referred to disease caused by Race 1, which is endemic in the southern US. A new classification system has been proposed.

Table 1. Races and Biovars of *Ralstonia solanacearum*

Race	Host Range	Geog. Distribution	Biovar
1	Wide	Asia, Australia, Americas	3, 4 1
2	Banana Other Musa spp.	Caribbean, Brazil Phillipines	1
3	Potato, some other Solanaceae, Geranium; plus a few other species.	Worldwide except US and Canada	2
4	Ginger	Asia	3, 4
5	Mulberry	China	5

(Reprinted, with slight modification, from Denny and Hayward, 2001)

Currently, the most important race/biovar of *R. solanacearum* is Race 3, Biovar 2, which has valuable agricultural hosts as well as a few known ornamental hosts. It is of special concern to the potato industry in the United States and Canada, because Race 3 has shown cold-temperature tolerance and has caused serious disease problems in potato crops in other temperate countries around the world. Bacteria identified as Race 3, Biovar 2 of *R. solanacearum* were imported into the US from Guatemala in geranium cuttings (Kim et al 2003) on several occasions in 1999 (Williamson et al 2002; Kim, 2002). Geranium industry leaders have since come together to discuss their clean stock systems and work with the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) and the Society of American Florists (SAF) to develop procedures that tightly guard against the introduction of any race of *R. solanacearum* via geraniums. Efforts made to improve the cleanliness of offshore geranium material were successful in 2002. There were no reported cases of *R. solanacearum* Race 3,

Biovar 2 in the US—and bacterial blight ... current 2003 outbreak of *Ralstonia* deriv... contamination of 7 stock plants. State an... disease that reached US growers.

Symptoms

Ralstonia solanacearum Race 1 is endemic in the United States, so growers may encounter this pathogen occasionally, particularly in the Southern US. This race is known to infect hundreds of different plant species in 50 families. Some of the flower crops known to be susceptible to Race 1 are browallia, ornamental peppers, catharanthus, cyclamen, dahlia, fuchsia, gerbera, hydrangea, impatiens, lantana, pelargonium, cineraria and schizanthus.

Because *R. solanacearum* causes a systemic vascular wilt and does not cause leaf spot symptoms, it does not appear to be very contagious within a greenhouse on overhead-irrigated crops. The route of infection is usually through the roots. In the operations that encountered *R. solanacearum* on their geraniums in 1999, none of the growers reported spread to any of their other flower crops, and symptoms did not appear to spread within the crop. The picture is quite different for subirrigated crops: *Ralstonia* spreads very easily via water used for subirrigation, just as it will spread rapidly by water in field situations. Irrigating geranium stock plants on trickle-tubes is an important safeguard against spread of either of the systemic bacterial diseases. Geranium st...

Importance

Symptoms:

The symptoms of a geranium with Southern wilt are sometimes very hard to distinguish from those of bacterial blight caused by *Xcp*. Both diseases cause a wilt of the systemically infected plants. *Xcp* can also cause leaf spots, but *Ralstonia* cannot. Plants infected by *R. solanacearum* will show yellowing, wilting and browning of lower leaves. If petioles of infected, symptomatic leaves are chopped up and set into water in a test tube, the water will often turn very cloudy as the bacteria stream out of the xylem. Vascular discoloration in the stem is common, and roots may sometimes turn brown. With *Xcp*, vascular discoloration is less pronounced or absent, and roots remain white.

Importance:

This disease is important to the flower industry because of its threat to geraniums and other ornamentals and also because of its potential threat to food crops. Fortunately, safeguards are already in place to protect geraniums against both diseases: ... effective against both *Xanthomonas* and ... growing practices at every greenhouse, th... geraniums are disease-free will keep gro... eradication effort will prevent *Ralstonia* ... this country.

References

References:

Denny, T. P. and Hayward, A.C. 2001. Ralstonia, pages 151-174 in: Schaad, N. W. et al. Laboratory guide for the identification of plant pathogenic bacteria, 3rd ed. APS Press, St. Paul, 373 pp.

Harris, D. C. 1972. Intra-specific variation in *Pseudomonas solanacearum*. Pages 289-292 in: Proc. Int. Conf. Plant Pathog. Bact., 3rd.

Hayward, A. C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev. Phytopathol. 29:65-87.

Hayward, A.C. and Hartman, eds. 1995. Bacterial Wilt: the disease and its causative agent, *Pseudomonas solanacearum*.

Protocol

Shipping

Examination

Storage

Screening

ial suspected of containing *Ralstonia*
tory.

1-Shipping:

Sample submission may be directly from a grower questioning the cause of symptomatic plants or from regulatory personnel that have reasons for suspecting a possible infection.

1. Suspect plant material must be placed in double ziplock bags and stored in a refrigerator awaiting shipment to a diagnostic facility. The preferred method for shipment is triple packaging. shipping container should be an approved uld be closed with approved shipping tape.

will place an identification card containing r code-three letter facility code-sample number) and a completed APHIS, PPQ form 391 inside the inner bag.

3. Samples should be shipped via overnight delivery.

2-Examination:

d within a certified biological safety cabinet. ng the examination must be separated and tified autoclave. The surface of all materials must be disinfested prior to the removal from the biological safety cabinet.

3-Storage:

While examination and testing is being conducted, suspect plant material and cultures must be stored in access controlled cabinets and/or refrigerators.

nded periods of time is not recommended. ribed in section #7, Sample Destruction. mission to your facility is no confirmation

4-Screening:

Any of the techniques listed below are recommended for determination of a possible *R. solanacearum* R3B2 infection. Other techniques may also be valid. However the use of a serological test kits for the identification to the species level is recommended prior to submission to another facility for identification to Race and Biovar.

a. Look for Bacterial Streaming. Clouds of bacteria should quickly form when petioles of the suspected plants are placed in tubes of sterile water. Note that this may only occur when viewing plant material with an advanced stage of infection.

b. Make isolations using a modified tetrazolium medium (TZC).

The bacteria may be cultured from diseased stem or petiole tissue in a diagnostic laboratory by streaking from the bacterial that flow out from diseased tissue into sterile water.

TZC medium is a modification of CPG medium and is similar to SM-1 medium in N. W. Schaad, J. B. Jones, and W. Chun (ed.), 2000, Laboratory Guide for Identification of Plant Pathogenic Bacteria, 3rd Ed. APS Press, St. Paul, MN.

Ingredients of CPG medium are:

5 g/l glucose
10 g/l peptone
1 g/l casamino acids
1 g/l yeast extract
18 g/l agar
pH to 7.0 with KOH as needed
autoclave (121C for 20-30 min)

To make TZC medium, allow the CPG to cool to approx. 55C, then add 2 ml of a filter sterilized 1% (10 mg/ml in distilled/deionized water) 2,3,5-triphenyl tetrazolium chloride (TZC) solution. Mix and pour plates immediately.

Ralstonia solanacearum appears as mucoid, whitish colonies that produce a reddish-pink diffuse pigment (it does not diffuse into the medium). Often there is a brown discoloration of the medium around the colonies. It's best to incubate at 28C to increase pigment production.

c. Identify to species using a Serological test kit. Below is a listing of USDA-APHIS-PPQ-CPHST approved serological test kits.

BID-*Rs* ImmunoStrip Test
Agdia, Inc.
30380 County Road 6
Elkhart, IN 46514
www.agdia.com
Phone: 800-622-4342
Fax: 219-264-2153

Potato Brown Rot Pocket Diagnostic
Central Science Laboratory (CSL)
Sand Hutton, York, YO41 1LZ
www.csl.gov.uk
Phone: 44 1904 462600
Fax: 44 1904 46211

Ralstonia solanacearum SPOT√CHECK LF™
Adgen, LTD.
Nellie's Gate, AYR
Scotland, KA6 5AW
www.adgen.co.uk
Phone: 44 1292 525275
Fax: 44 1292 5255477

Communication

If a positive result of *Ralstonia solanacearum* is received from the serological test kit, the sample (plant material or culture) must be submitted to USDA-APHIS-PPQ-CPHST for confirmation to race and
s to receive samples submitted for

BARC-East, Bldg. 580
Powder Mill Road
Beltsville, MD 20705
Phone: 301-504-8141

Write the responsible diagnostician's name and contact information on the APHIS Form 391. Follow the shipping protocol (#1) when sending plant material to another diagnostic facility.

5-Communication:

If the serological test kit produces a **positive** ID to species, follow this communications protocol. If a **negative** result is produced, no further communications are necessary.

a. Notify Dr. Levy's lab of the suspect sample being shipped to their facility.

b. Contact the State Plant Health Director (SPHD) in the sample state of origin.

State Plant Health Director: _____
Address: _____
Address: _____
Phone Number: _____
Fax Number: _____
Email: _____

and fax the SPHD at the PPQ regional office:

1. A copy of the updated form 391 with the preliminary diagnosis and the responsible diagnostician's contact information,
2. a copy of the overnight delivery form used to submit the sample to Dr. Levy's Lab,
3. and a copy of the state inspector's sample card information submitted with the sample.

c. Notify your Institution's Environmental and Health Safety Official.

EHS Official: _____
Address: _____
Address: _____
Phone Number: _____
Fax Number: _____
Email: _____

d. Notify your Regional Center and other diagnosticians within your state, of your findings to this point. Please be very clear with the extent of the diagnosis, e.g. "*Ralstonia solanacearum* was detected on Geraniums using an Agdia Immunostrip test kit. This test only provides identification to the species level. The sample has been sent to Beltsville for confirmation to the race and biovar level." Do not include the submitter's name or contact information.

**National and regional network members will be notified by their Regional Center when confirmation is received from Beltsville.

Confirmation

6-Confirmation:

a. Diagnosticians will be notified of the results by Dr. Laurene Levy's laboratory.

b. Notify your Regional Center of the confirmation. The Regional Center will notify other Regional Centers and NAPIS.

Regional Center Contact: _____
Contact Phone Number: _____

c. States officials will be notified of the results by the PPQ regional office. Once confirmation is made, state and federal regulatory officials will handle any actions dealing with containment and eradication.

d. In compliance with the Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331), if a diagnostic laboratory held back part of a sample or culture that was later shown to be *Ralstonia sola*
required to notify,

APHIS, PPQ, Biolo
734-7211, 6828, or

Sample Destruction

as soon as possible that the
place within seven (7) days
Officer must have the oppo
time, or if the sample/culture has already been destroyed, the responsible laboratory manager/plant pathologist must complete the **APHIS form 2040** (Guidance Document for Reporting the Identification of a Select Biological Agent or Toxin in a Clinical or Diagnostic Laboratory) and sent to the permit unit in Riverdale, MD. This form is available on the Permits website at: http://www.aphis.usda.gov/ppq/permits/agr_bioterrorism/index.html

7-Sample Destruction:

Plant material, cultures and/or supplies used in the examination and isolation of the suspect sample must be destroyed using a biologically monitored autoclave. The autoclave must be set at a minimum of 15 psi, 121 C for 20 minutes.

Autoclaves are required to be testing periodically for their effectiveness. This can be achieved using a biological monitoring product. Information on one such product can be found at: http://cms.3m.com/cms/US/en/2-21/cirFFFQ/view.jhtml.

klsc 06/03

Commonly known as…

Host Range

Symptoms

Images

Ralstonia solanacearum Race 3 Biovar 2

Commonly known as:

Symptoms:

- Infected plants may appear asymptomatic until temperatures reach 63°F (17°C)
- Symptoms can easily be confused with Bacterial Blight caused by *Xanthomonas campestris* pv. *pelargonii*.
- Leaves appear diffusely chlorotic as opposed to vein bounded, v-shaped wedges of *Xcp* infections (figure 1).
- Lower leaves are the first to turn yellow, then brown.
- Chopped up petioles of symptomatic geraniums will produce a characteristic cloudiness

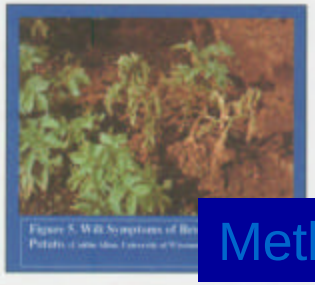
oor root development (figure 3).
tions (figure 2).

Images:

Other crops

Method of Diagnosis

Disease in other crops:



Method of Diagnosis:

- BID-Rs ImmunoStrip for Rs from Agdia, Inc., Potato Brown Rot Pocket™ Diagnostic from Central Science Laboratory, or *Ralstonia solanacearum* SPOT √Check LF™ from Adgen, Ltd. are recommended for identification to the species level.
- Identification to Race and Biovar must be conducted by Dr. Laurene Levy, APHIS- PPQ-CPHST, Beltsville, MD.

On-line resources

Select agent pathogens:

Pathogen:	**Disease:**	**Common Host:**
Phakopsora pachyrhizi	**Soybean Rust**	**Soybean**

USDA Protocols Soybean Rust: http://www.aphis.usda.gov/ppq/ep/soybean_rust/
APS Feature Story: http://www.apsnet.org/online/feature/rust/
North Central Soybean Research Program: http://www.planthealth.info/rust/rust.htm
North Central Pest Management Center:
http://www.ncpmc.org/soybeanrust/SoybeanRustPestAlertMay2003.pdf
Ohio State University Factsheet: http://ohioline.osu.edu/ac-fact/0048.html
Florida State University Factsheet:
http://www.doacs.state.fl.us/~pi/enpp/pathology/soybeanrust.html
US Soybean Diagnostic Guide:
http://www.unitedsoybean.org/soybeanrustguide.pdf

Plum pox potyvirus	**Plum pox**	**Stone fruits**

UDSA/ELISA Protocols: http://www.aphis.usda.gov/ppq/plumpox/protocols.html
A Plaque upon the land. http://www.aginfo.psu.edu/psa/sf2000/plague.pdf
USDA Information Sheet: http://www.aphis.usda.gov/ppq/plumpox/
APHIS page: http://www.aphis.usda.gov/ppq/ispm/nematode

Provide secured communications & tracking (IT Committee)

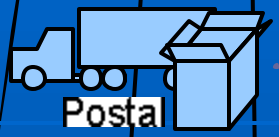
- Data and sample movement
- Secured communications
- Chain of custody

National Plant Diagnostic Network

Diagnostic Sample Check In

Client

Agent



Postal Service

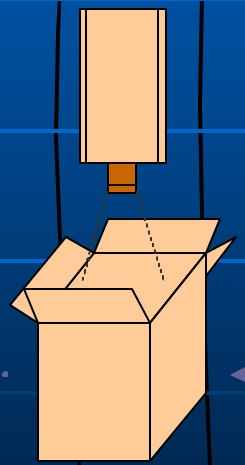
Sample Check-In

Mail-Ins

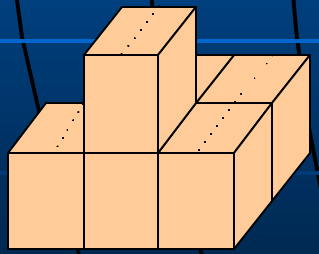
Walk-Ins

Scan Sample Barcodes from Request Sheet

Print Labels for Sample Tracking



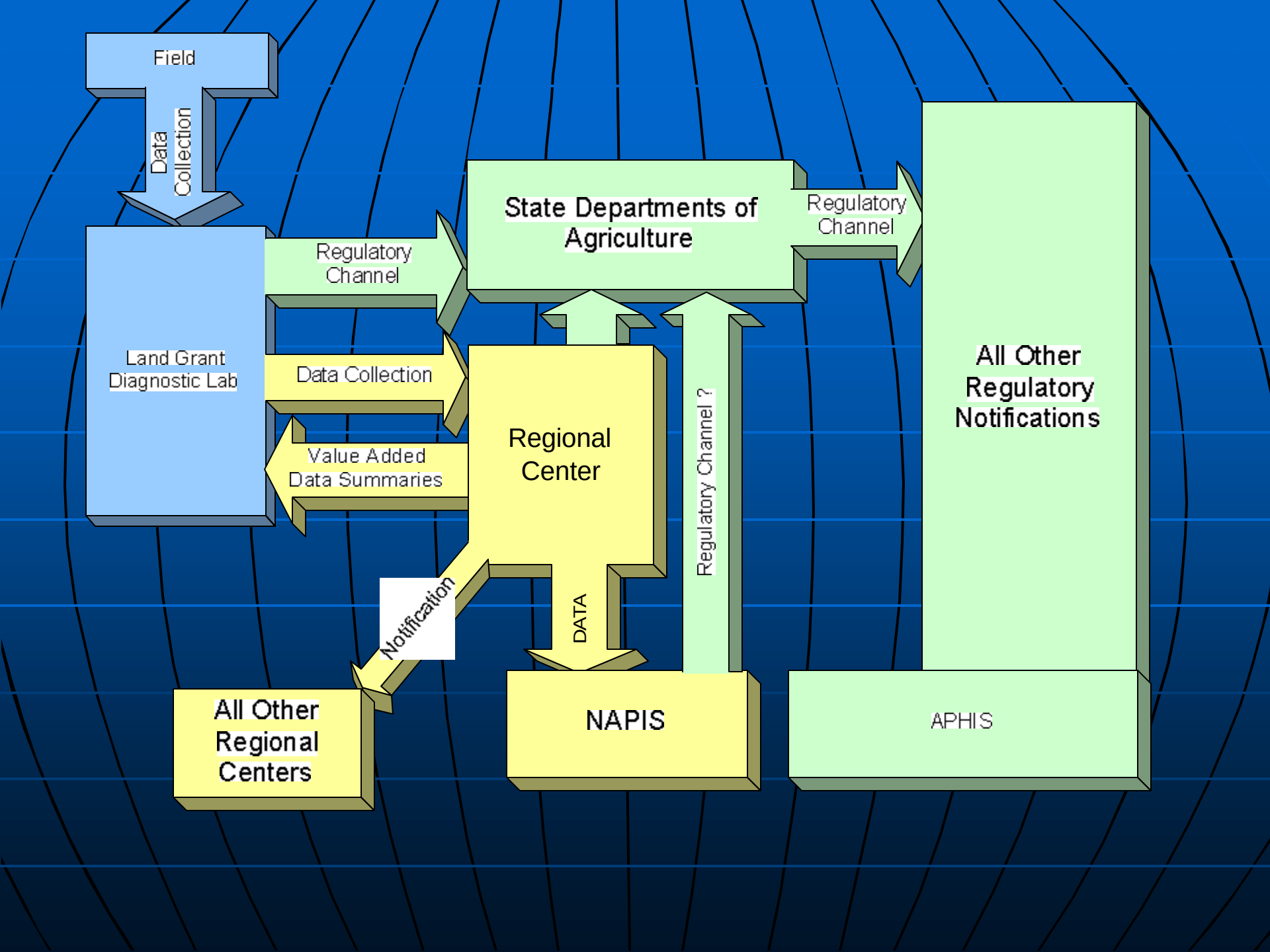
Photograph for records



Freezer Storage

Key elements of incident reporting

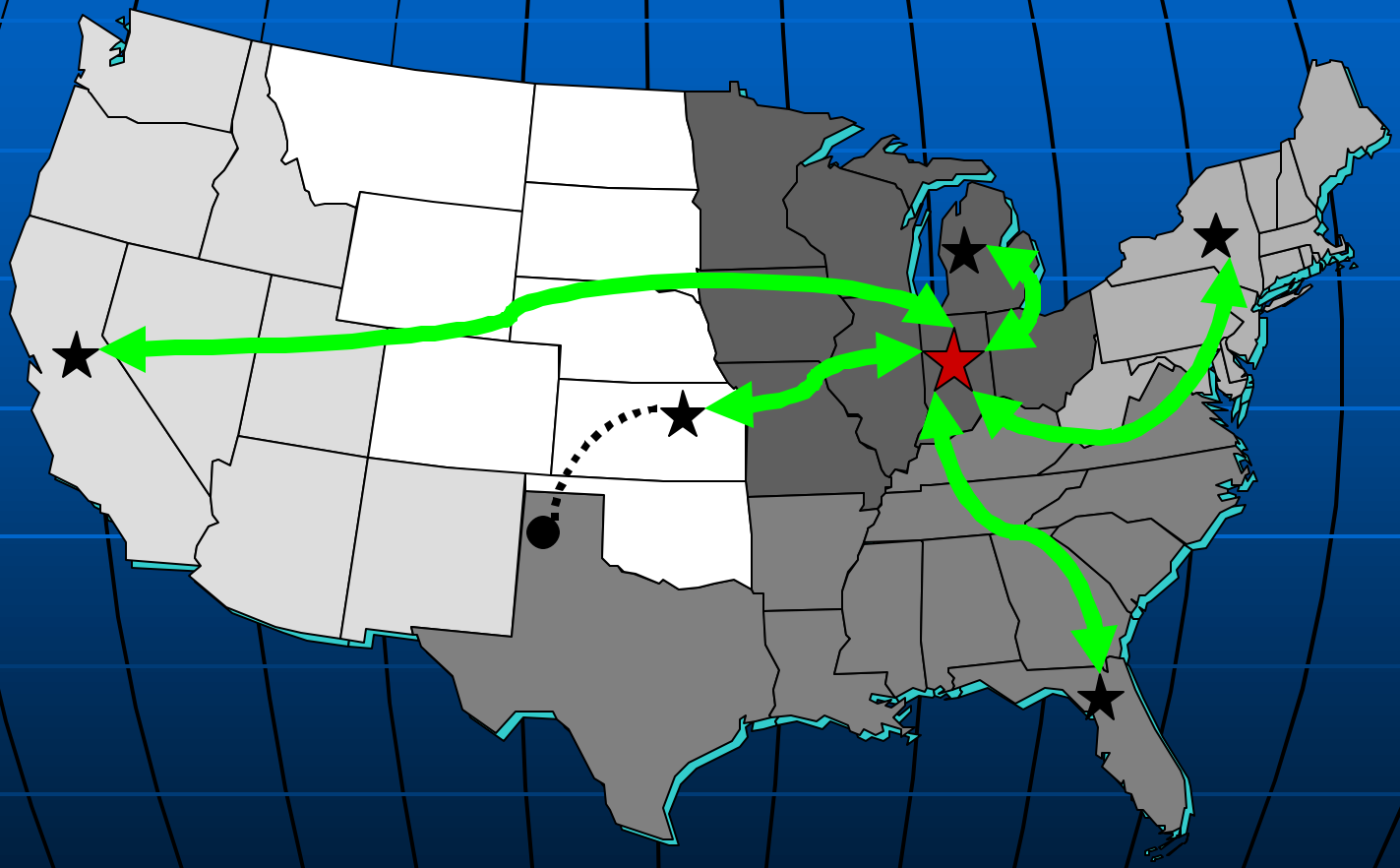
- Establish a clear reporting chain with receipt and acknowledgement criteria.
- Sensitive information will be sent in a secured manner to the appropriate contacts.
- A log of the notification system will be maintained to determine who has been informed, who has not been informed, and when they were informed.


Field
Data Collection
Land Grant Diagnostic Lab
Regulatory Channel
State Departments of Agriculture
Regulatory Channel
All Other Regulatory Notifications
Data Collection
Value Added Data Summaries
Regional Center
Regulatory Channel ?
Notification
DATA
All Other Regional Centers
NAPIS
APHIS

Regional diagnostic centers serve as central point for information flow

Michigan State University Regional Plant Diagnostics Ctr.

Regional Diagnostic Centers upload data to NAPIS



Create a national database to monitor disease and pest outbreaks (Data Process Committee)

- National Agriculture Pest Information System (NAPIS)

National Plant Diagnostic Network

Data processing will provide

- Summary reports
- Distribution maps
- Large collection of biological images
- Pattern analysis
- Data sets for use in other studies

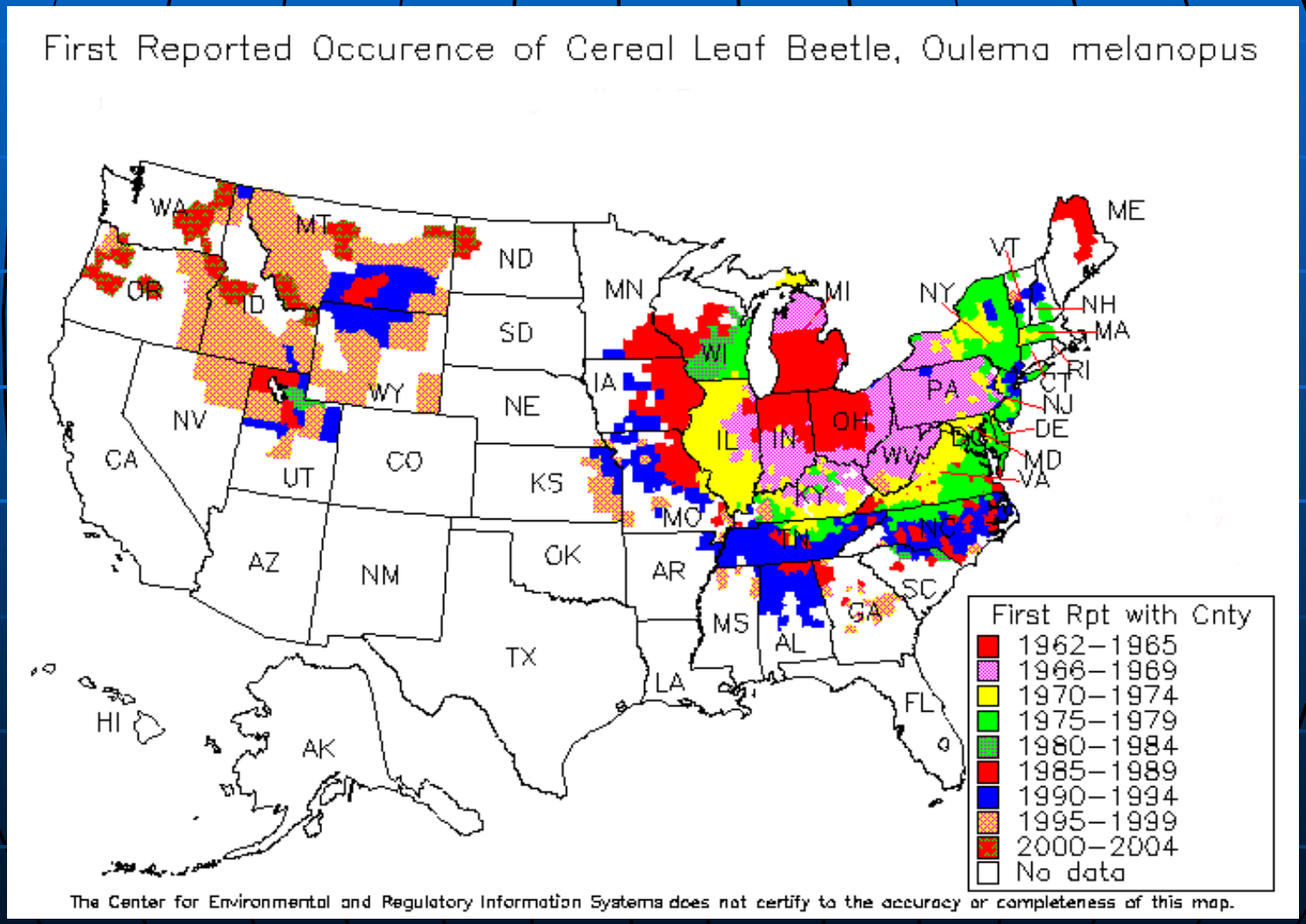
National Plant Diagnostic Network

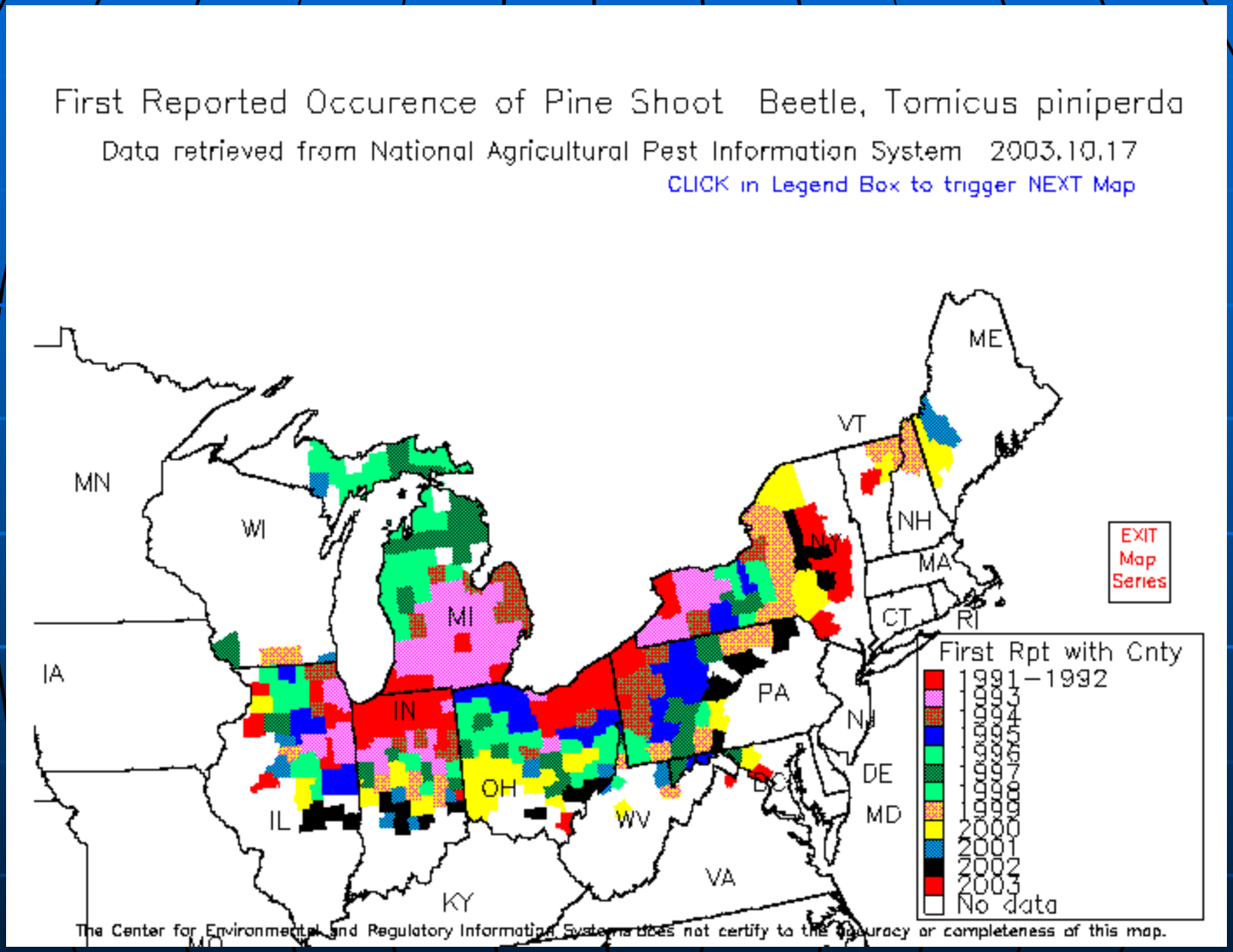
Create a data analysis system to detect anomalies (Data Analysis Committee)

Goal: To establish an analysis system that improves our ability to detect an introduction of a biosecurity threat to agriculture, whether intentional or not, using diagnostic clinic records

National Plant Diagnostic Network

For example: NAPIS CAPS Data 1962-2003 Animation Loop – Cereal Leaf Beetle shows Jump in Distribution – Why?




First Reported Occurence of Pine Shoot Beetle, Tomicus piniperda
Data retrieved from National Agricultural Pest Information System 2003.10.17
CLICK in Legend Box to trigger NEXT Map
ME
VT
NH
MA
CT
RI
NY
MN
WI
MI
IA
IN
OH
IL
PA
NJ
DE
MD
WV
VA
KY
EXIT Map Series
First Rpt with Cnty
1991-1992
2000
2001
2002
2003
No data
The Center for Environmental and Regulatory Information Systems does not certify to the accuracy or completeness of this map.

NPDN Scenario Training

Carla S. Thomas, University of California, Davis

- Objective
  - Practice operational functions of NPDN in a non-emergency environment
  - Relationship Building
  - Communications Facilitation
  - Engagement of other detection/response entities and agencies
  - Improvement of protocols and processes

Who Participates?

- NPDN diagnosticians
- Regional NPDN Director, National Prog. Leader
- APHIS State Plant Health Director (State SPHD)
- State Plant Regulatory Officer (State SPRO)
- Crop specialists (University Experts and County Agents, IPM and Extension)
- APHIS National Identification Service Designates
- Industry
- Others

Examples

NPDN Exercise Evaluation - Microsoft Internet Explorer provided by MSN

File Edit View Favorites Tools Help

Back Forward Stop Refresh Home Search Favorites History Mail Print Edit Discuss MSN Messenger

Address http://www.pdis.org/ExerciseScenarios/Scenarios/01_SoyBeanRust/report.aspx

Go Links

Event: 6/11/2003 6:05:00 PM UTC [Reported: 6/11/2003 12:09:47 PM CST]

UNL Diagnostic Lab

I called Vicki Wohlers, state SPRO, to inform her of the suspect soybean rust sample that came into the UNL diagnostic lab this morning. I notified her that a sample will be sent to the diagnostic lab at KSU and also to the APHIS lab in MD.

- Jennifer Chaky

904: Receipt

Event: 6/11/2003 6:30:00 PM UTC [Reported: 6/11/2003 1:10:03 PM CST]

Nebraska Department of Agriculture, Lincoln, NE

Received call from Jennifer Chakey, UNL Plant Pathology diagnostician, at approx. 11:50 am who informed me that a suspect soybean rust sample had been received at the Triage Lab. She informed me that the sample would be sent onto the Expert Lab and also to the APHIS Confirming Diagnosis Designate. I also got information from Jennifer such as: who brought the sample in, location information on the field such as county, section, township, range and/or GPS reading. I then followed protocol (what we will do if sample is positive) for containment and delimitation of the site in coop. w/Steve Johnson, SPHD.

- Vicki Wohlers

911: Receipt

Event: 6/11/2003 6:00:01 PM UTC [Reported: 6/11/2003 1:30:03 PM CST]

Lincoln

I recieved a call from Jennifer Chaky about possible soybean rust in sample collected by Jim Stack. Questions that I have is that I would like to have more information on the location of the sample, what county did it come from, amount of acres in field. I believe we need to be prepared to respond to this sample maybe delimit the area.

- Steve Johnson

918: Notification

Event: 6/11/2003 8:30:00 PM UTC [Reported: 6/11/2003 2:32:46 PM CST]

APHIS Beltsville

I called Bob Spaide (who had just landed in Arizona) to tell him of the suspect that was being sent to me as part of the simulation. I should have called him earlier (and I imagine I would have had it been the real thing but thats no excuse!).

- MaryPalm

925: Notification

Event: 6/11/2003 3:30:00 PM UTC [Reported: 6/11/2003 2:47:27 PM CST]

UNL Diagnostic Lab

I received a call from Jim Stack, UNL cooperative extension staff, to notify me that he was going to be delivering a sample to the UNL diagnostic lab which he believed may be soybean rust.

Done Internet

Summary

Agriculture is an easy, accessible target. To protect it we need:

- Communications and reporting infrastructure
- Data management and a national data base
- Real-time pattern analysis capacity
- Trained diagnosticians and detectors
- Both survey and surveillance to monitor not only food crops but ornamentals, turfgrasses, and forests.

Questions?

National Plant Diagnostic Network

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